

**Automatic valve (cock) mixing instrument
user manual.
(including X-ray scattering mixer supplement)**

Unisoku, Co. Ltd.

Translated by H. Tsuruta

This manual describes our standard stopped-flow instrument. Some diagrams and figures may not apply to the stopped-flow instrument for x-ray solution scattering.

Unisoku.
2nd technical division.
Unisoku Co. Ltd.

Automatic valve (cock) mixing instrument user manual.

Unisoku.

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p1.

1. Introduction

Thanks for purchasing a Unisoku product. This manual describes the function and operation procedure of the auto-valve stopped-flow mixer as well as its accessories. We reserve the right to alter the content of this manual, names, hardware specifications, application software. Please contact us should you require clarification.

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2. Specifications

Auto-valve mixing instrument

standard sample reservoirs: 10mm ID, 40mm long, 3.14ml capacity

driving mechanism: gas pressure driven pneumatic syringe

flow path valve: gas pressure driven auto-valve

sample syringes: 5.7mm ID, 10mm total maximum travel,

0.25ml max. capacity

mixing cell: ceramic, 2-jet

observation cell: 10mm light path, 2mm ID, quartz window

N₂ gas pressure (recommended) 0.3-0.6 MPa (3-6 kg/cm²)

mixing dead time: 1.5ms or shorter

observation modes: absorption, fluorescence (optional)

temperature range: 10-50 C

tubing for water circulation 6mm OD polyurethane tubing (for temperature control)

air syringe tubing: 4mm OD polyurethane tubing

actuator tubing: 4mm OD polyurethane tubing

delay timing for mixing three solutions: 30ms - 10s

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3. operation principle

The stopped-flow technique is one of the chemical relaxation techniques.

A pair of fast flowing solutions is mixed together rapidly to initiate a reaction. Then, the flow is stopped, the progress of the chemical reaction is monitored by an optical technique. The mixing instrument (stopped-flow mixer) has a mixing dead time shorter than 1.5ms, which limits the shortest reaction time constant to be observed effectively.

The flow passage is designed to withstand the high pressure of the flowing sample solution.

The instrument has a monolithic construction, containing sample reservoirs, syringes, mixer and observation cells in order to maintain sample temperature constant and to reduce sample volume.

The syringes are driven by a pneumatic air cylinders. A separate pneumatic air cylinder drives the flow valve, which switches the connection of the syringes to either the reservoir or the observation cell.

When a "SET" operation is achieved, sample solutions are loaded into the syringes. A "MIX" operation will mix two sample solutions, sending the mixed sample into the observation cell via the mixing cell.

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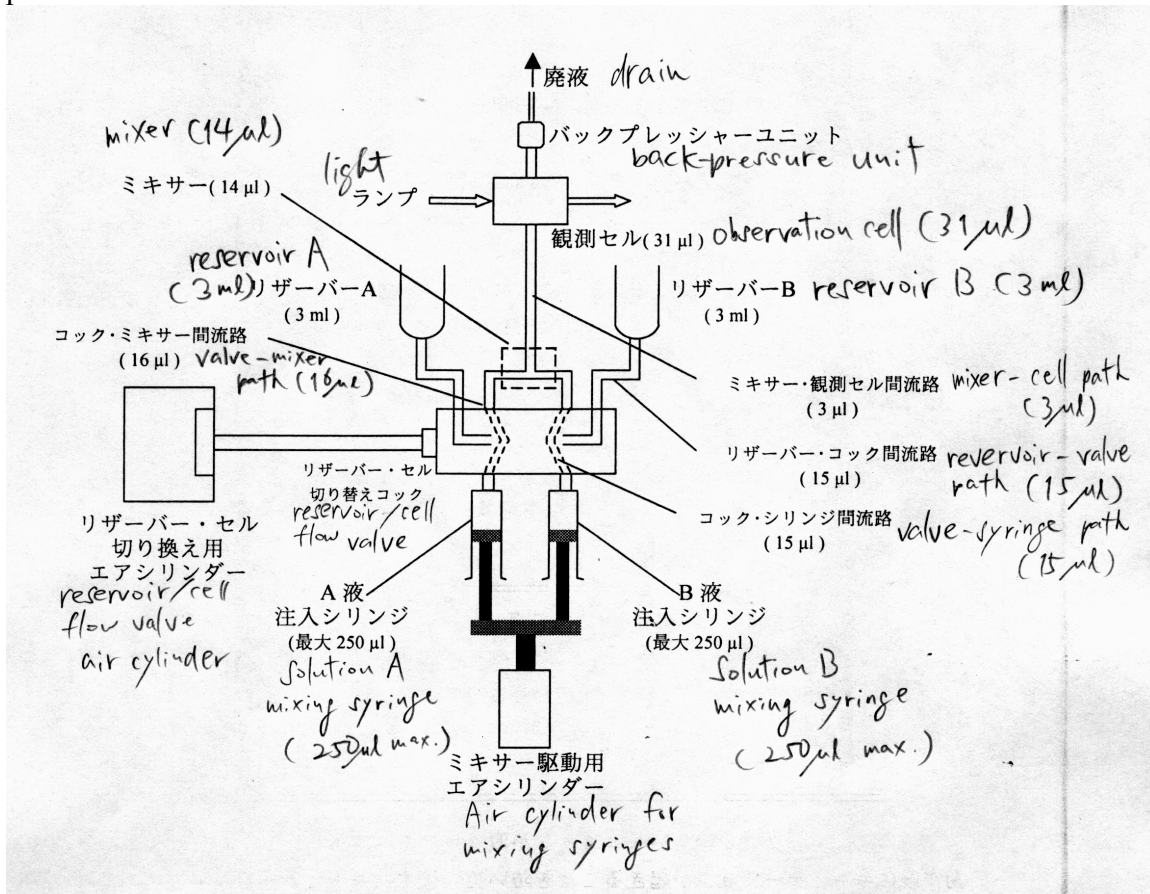
SET performs the series of events: switching flow valve, pull the syringe plungers and load aliquots of sample solutions from the reservoirs into the syringes, switching back the flow valve. MIX pushes the syringe plungers to rapidly transfer sample solutions into the observation cell via the mixing cell.

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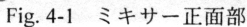
The drain outlet is equipped with a back-pressure unit, which prevents "cavitation effects" (The rapidly flowing solution produces negative pressure downstream after passing the observation cell. This sometimes produces small bubbles in the the observation cell. This phenomenon is called "cavitation effects".)

The sample cell has quartz windows on the front and back sides.

Fig. 3-1 shows the flow diagram.



Mixer	Lamp	Back pressure unit
	Reservoir A (3ml)	Observation cell (31 microliter)
Valve contact surface to mixer		Reservoir B (3ml)
	Reservoir-obs.cell switching valve	Path (mixer – obs. cell) (3 μ l)
Reserver-cell switching air cylinder		Path (reservoir – valve) (15 μ l)
	syringe A (250 μ l max)	Path (valve – syringes) (15 μ l)
		syringe B (250 μ l max)
	Air cylinder for mixing	



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Back-pressure unit: to prevent "cavitation effects"

Light guide connection (IN): connect a light guide for a light source

Light guide connection (OUT): connect a light guide for spectrometry

Flow valve: switches between (syringe-reservoir) and (syringe-mixer/obs. cell).

Connections for circulating water: for temperature control. Use 6mm OD polyurethan tubing.

Gas flow tube: Use 2mm OD Teflon tubing.

Pneumatic actuator for mixing: SET loads sample solutions from reservoirs and performs PUSH (MIX) to send sample solutions in the syringes to complete a mixing event.

Air tube connector for mixing: The upper connector is for "SET" and the low for "MIX". Use 4mm OD polyurethan tubing. The upper connector for "SET" has a air speed controller. one can adjust Pull speed upon SET motion with this controller (See Chapter 9 for details). Equipped with contact sensors on its/their front (See Chapter 8 for details).

Flow valve actuator: operated automatically.

Air tube connection for the flow valve: Use 4mm OD polyurethan tubing. Each has an air speed controller. One can adjust the speed of the valve switching with this controller (See Chapter 9 for details).

Air speed controller: One can adjust air intake speed.

Syringes: Sample solutions are loaded into these upon SET motion. MIX motion mixes the two solutions in the syringes.

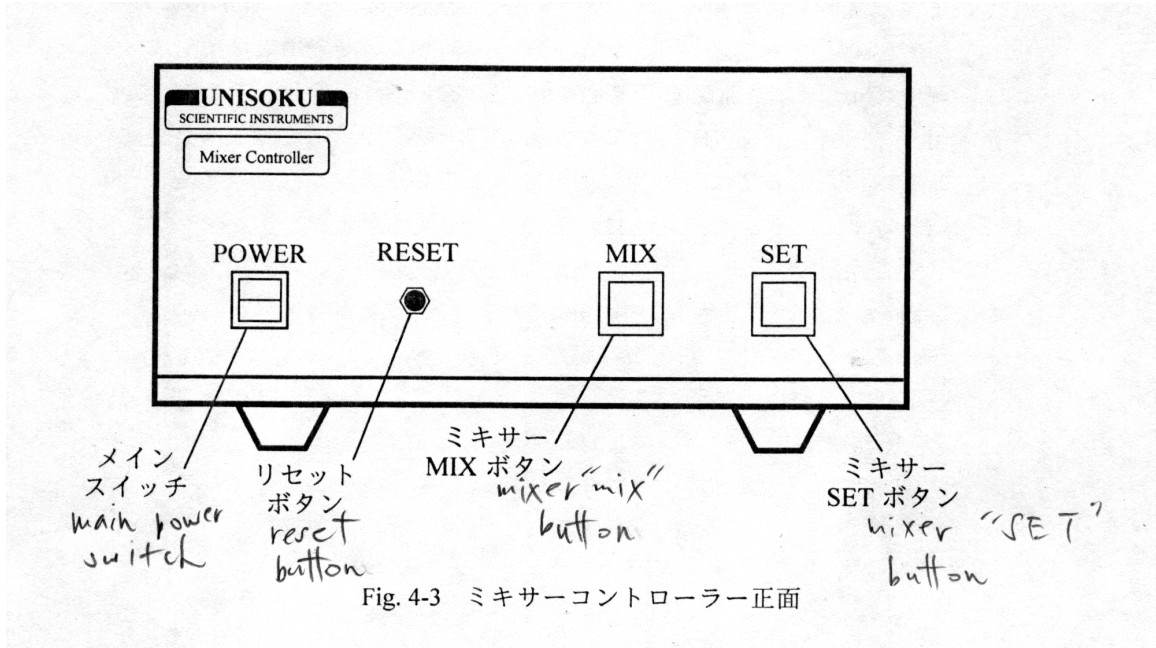
Flow valve contact signal cable: (self explanatory)

MIX contact sensor: senses the mixer cylinder MIX status.

SET contact sensor: senses the mixer cylinder SET status.

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- Solution flow passages, reservoirs, syringes, mixer and observation cell can be maintained at a constant temperature by circulating water of constant temperature. 10-50C is the recommended temperature range.
- Light guides should be connected to specified light guide ports. Light guide IN should be connected to a light source, and Light guide OUT should be connected to a spectrometer. One end of a light guide has a round cross-section and the other a line. We recommend connecting the round cross-section to the stopped-flow mixer. A light guide should be connected to the fluorescence light guide connection for fluorescence measurements.
- The previously mixed solution comes out of the drain outlet, which terminates as a 50cm Teflon tube, which should be secured to a separate container to receive the mixed sample solution.
- The sample reservoirs have caps, which help prevent clogging of flow passages due to dust.
- The instrument has the provision of putting sample solutions under an anaerobic condition. (This is not important at this point of time.)



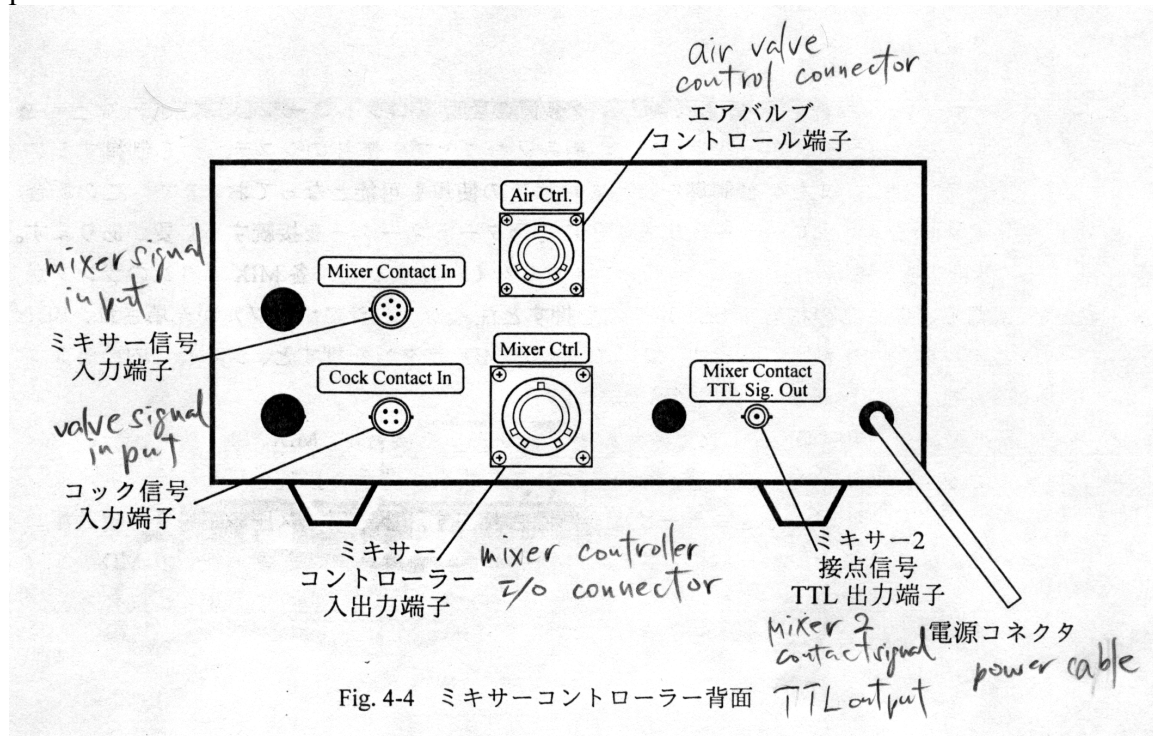
Main power switch Reset button Mixer "Mix" button Mixer "Set" button

main power switch

reset button: reset errors. Forces cancellation of motions.

mixer MIX button:

mixer SET button:



flow valve input connection

mixer signal input

air valve control connection

mixer controller inputs: used with the UNISOKU main controller to operate the mixer. Connect a terminator

when the main controller is not used.

mixer contact signal TTL output: upon a completion of sample flow (completion of

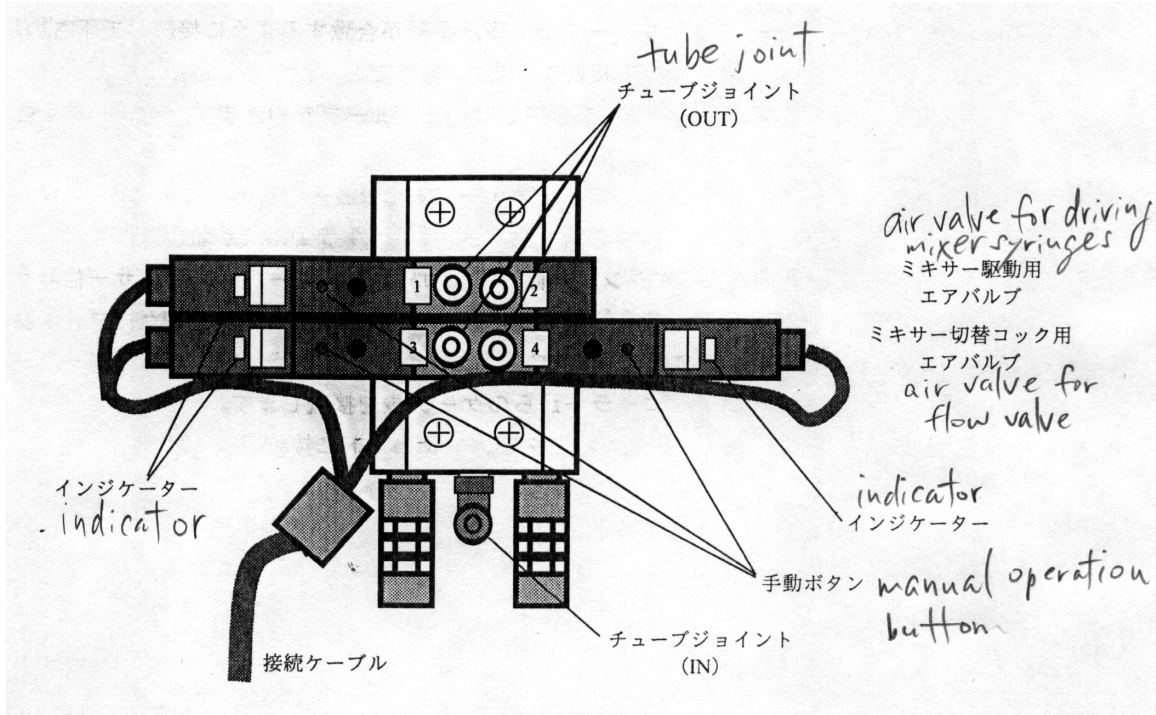
mixing) a negative TTL pulse (~1ms width) is generated. The polarity of this pulse can be reversed by a jumper switch inside the controller.

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- The mixer controller 'controls' the mixer motions.
- This mixer can be integrated to other UNISOKU system and can be controlled by our other instrument. It can also be used as a stand-alone system. A special terminator is required for the latter mode of operation, however. (Disregard this description.)
- Complete backside connections, then turn on main power. This should turn on "MIX" button indicator light. Pressing "SET" button will load syringes with sample solutions and turn on "SET" button light. While "SET" button is lit, press "MIX" button to perform rapid mixing of the sample solutions.
- When there is a poor electrical connection upon turning on main power "MIX" and "SET" buttons will lit alternatively to notify the error. Correct poor connection(s) and push the reset button for one second or longer to cancel the error state. Lower pneumatic cylinder gas pressure misaligned air cylinder sensor positions may make "MIX" and/or "SET" button temporarily non-functional. Pressing the reset button will halt the current motion and allows to move on to next motion.
- Please verify for the MIX status before the main switch is turned off. The controller is forced to b in MIX status when the main power is turned off in SET status.

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Air valves.



(diagram)

Air valve for mixer ----- controls air cylinder for syringe plungers

Air valve for flow valve ----- controls air cylinder for the flow valve

Tubing joint (IN) ----- Supply a pressurized air or gas using a polyurethane tubing

Tubing joint (OUT) ----- These should be connected to the pneumatic cylinder, actuators.

Indicators ----- Lits when air valve is in operation.

Manual button ----- Allows manual operation of air valves

Cables ----- These are used to connect to the mixer controller.

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5. Tubing/cable connections.

(1) Connect black polyurethane tubes so that indicator numbers match. Note that inserting a tube is sufficient to make complete seal. It is important to insert all the way in. Depress the black ring and gently pull the tube to disconnect.

(2) Connect the mixing valve signal cable and the mixer signal cable to the corresponding receptacles on the back side of the controller box. (There are two cables.) Also connect air valve connection cable. When this instrument is used along with other UNISOKU system, a cable from the stopped-flow controller should be connected to the UNISOKU main controller. Finally, connect the power cable.

6. Start-up and power-down procedure

Start-up.

- (1) Make it sure that all cables and tubes are properly connected.
- (2) Open the gas bottle main valve and adjust the pressure regulator to 0.3-0.6MPa. We recommend 0.5MPa.
- (3) Turn on the UNISOKU main controller when you use the stopped-flow instrument with another UNISOKU unit. If you operate the stopped-flow as a stand-alone instrument, skip to (4).
- (4) Turn on the stopped-flow controller power switch.

Power-down

- (1) Rinse well before turning off. First, one should remove remaining sample solutions from the reservoirs. Fill the reservoirs with distilled water (or appropriate solution) and rinse internal components by pressing SET and MIX repetitively. Rinse very carefully especially after using organic solvent. Acidic and alkaline solutions can be washed out easily with distilled water.
- (2) Leave both syringes in MIX state. It is recommended to have the reservoirs filled with distilled water for long-term storage.
- (3) Turn off the mixer controller power.
- (4) Turn off the main controller power when you use this instrument along with another UNISOKU unit. (Skip this step when you use the stopped-flow instrument as a stand-alone instrument.)
- (5) Shut off the main gas supply valve.

7. Recording.

- (1) first circulate water whose temperature is regulated in order to control sample temperature.
- (2) Put sample solutions in the sample reservoirs.
REMARK!: Do not use a glass pipette or similar objects to place sample solutions as broken fragments can clog the sample passage. Protect the tip of the glass pipette by connecting a short piece of silicon tubing if you must use glass pipette.
- (3) Place an appropriate combination of spacer on top of the pneumatic cylinder to adjust the amount of stroke (sample volume to be mixed).
- (4) Wait until sample temperature stabilizes.
- (5) Input parameters in the Unisoku software if the stopped-flow is controlled by a Unisoku system. When it is used as a stand-alone equipment, do the following:
 - Press "SET" button to load sample solutions into the sample syringes.
 - Press "MIX" button to initiate rapid-mixing for a set of measurements.
- (6) Upon the completion of the mixing event, repeat (5) if you are using the same samples. When you switch to different sample(s), first remove the sample solution(s) and rinse the reservoir well. Fill the same reservoir with a buffer solution or a sample solution and repeat (5) three times. Follow (2)-(5) for a next set of measurements. Refer to Chapter 6 for concluding measurements.

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8. Flowing argon gas (optional)

One can introduce argon gas for conducting measurements under anaerobic conditions in the following way:

- (1) Adjust argon gas pressure to 0.3-0.6MPa (0.5MPa recommended).
- (2) Attach a Teflon tube into the sample reservoir through the reservoir cap. Argon gas can now be blown into the sample directly.
- (3) Blow argon gas near the bottom parts of the syringes in order to prevent air from entering into the syringes via the plunger gap.

9. Adjusting contact detection sensor

This instrument is equipped with two types of sensors that detector status of the pneumatic cylinder. There is one on the side and the other in the back side of the pneumatic cylinder. These sensors detect “MIX” and “SET” status, respectively. The placement of these sensors have been adjusted upon shipment of the mixer. (probably there is no need to readjust.) It is, however, possible to begin recording immediately before the completion of an mixing event by adjusting the location of the “MIX” sensor.

Adjusting MIX sensor.

- (1) Press “MIX” button to assure the pneumatic cylinder is in “MIX” position (upper position).
- (2) Loosen the small screw that holds the MIX sensor on the side of the pneumatic cylinder.
- (3) Slid up the sensor gradually along the groove from the lowest position. Observe the small light on the sensor comes on and off when the sensor is moved up further.

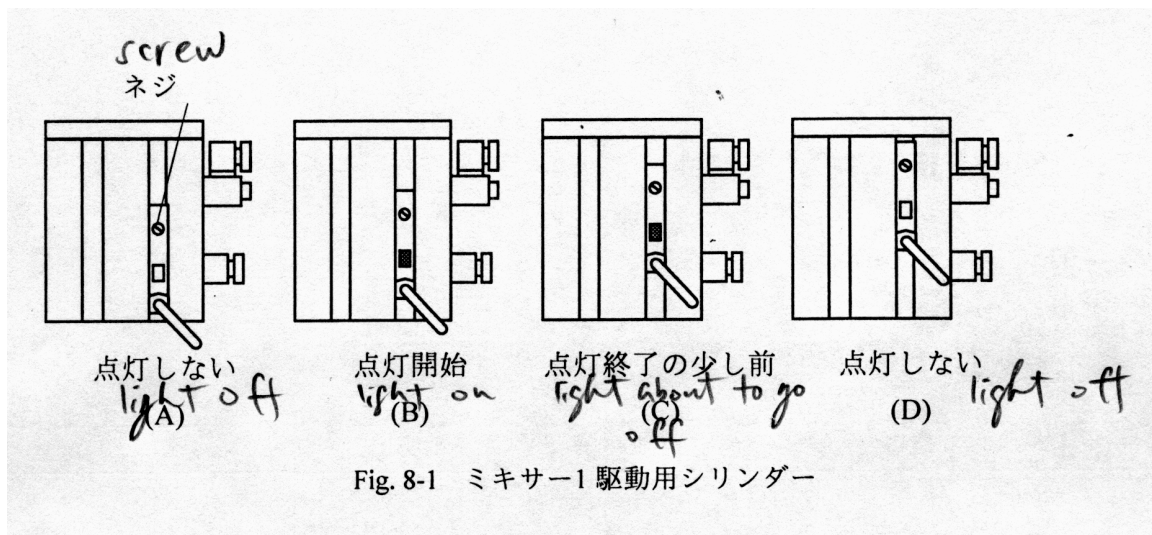


Fig. 8-1 ミキサー1 駆動用シリンダー

Air cylinder for mixing

(A) no light; (B) light on; (C) Immediately below the position where the light turns off; (D) no light.

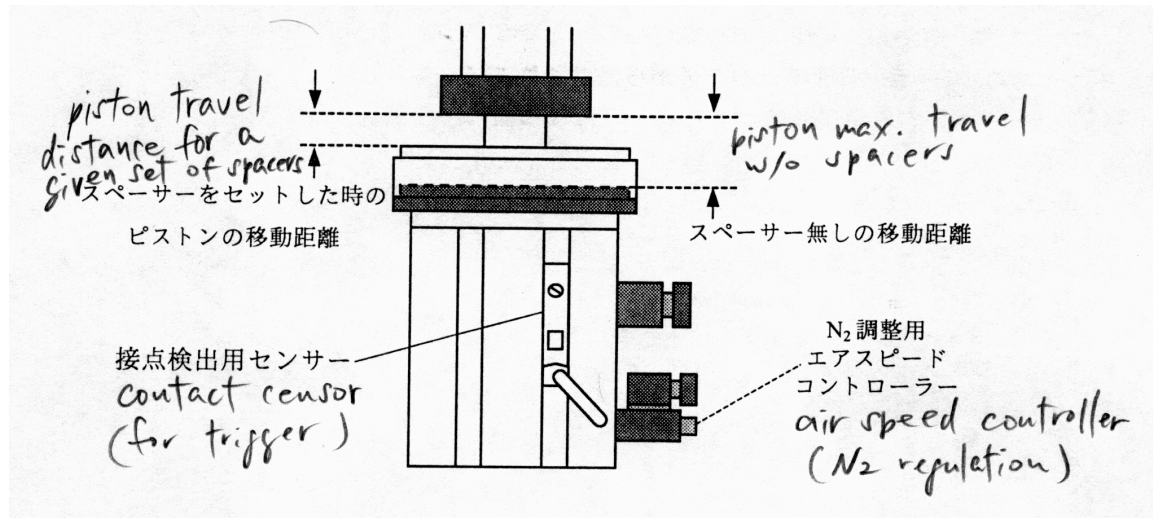
- (4) Tighten the screw at the lowest position where the sensor light remains off just above the range in which the light comes on.
- (5) Press “MIX” and “SET” buttons step by step to verify the sensor light comes on and off in a fashion outlined below:
 - Press “SET” button and confirm that the “SET” sensor light turns off as soon as the pneumatic cylinder starts to move (down).
 - Press “MIX” button and confirm that the “SET” sensor light comes on at the end of “MIX” motion.

The trigger for initiating measurement is sent by the center sensor of the Mixer 1 pneumatic cylinder (???). Therefore, one can adjust this timing by setting the sensor at different locations. For instance, a location between (B) and (C) would give a trigger pulse before the completion of mixing so that one can measure signal before mixing.

A connector on the back panel of the controller gives 0 or 5V when the sensor is on or off, respectively.

NOTE by HT: it is unclear which sensor is supposed to be which one. Some experiments are needed to clarify.

10. adjusting mixing volume and sample loading speed



Piston travel with a set of spacers installed

Air cylinder sensor

Piston travel w/o spacers

N2 flow speed controller

Mixing volume:

- (1) Put the pneumatic cylinder into "MIX" status.
- (2) Insert a desired combination of spacer on top of the pneumatic cylinder. The mixer was shipped with three spacers of different thickness (1,2 and 3mm each).

Sample loading speed:

This is adjusted by the needle valve at the bottom of the pneumatic cylinder. It is recommended to adjust this so that sample loading takes 4-5 seconds when all spacers are removed. Formation of bubbles in the sample solutions can result otherwise.

Changing mixing ratio

This is achieved by employing different syringes. The mixer is usually shipped in the standard 1:1 mixing ratio configuration. We have different syringe combinations for 1:4, 1:6 and 1:9 (optional).

The high mixing ratio can result in poor mixing efficiency, depending on solvent. In this even the mixing chamber should be replaced (with that of higher mixing efficiency).

Available mixing ratios, syringe internal diameter and compatible O-rings are tabulated below.

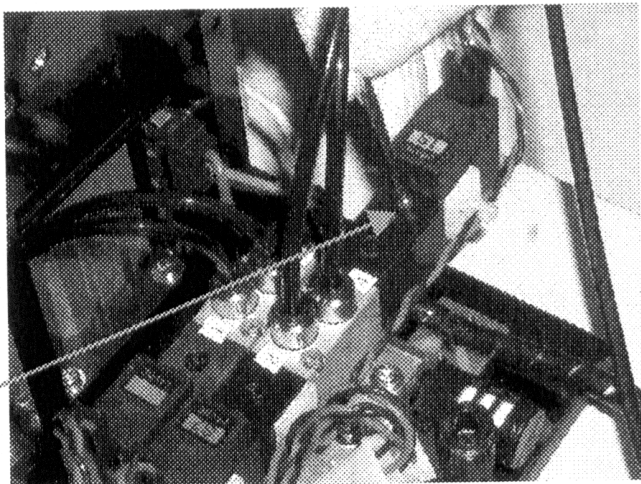
Ratio	1:6		Inner diameter (mm)	O-ring type
1:1	1		5.7	S-3
1:4	1		3.26	3.2*2.1
	4		6.6	S-4
1:6	1		3.26	3.2*1.2*1
	6		8.0	P-5
1:9	1		2.2	N/A
	9		6.6	S-4

Refer to the next page for changing syringe(s).

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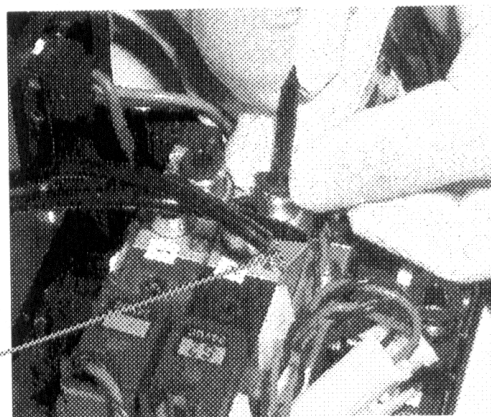
- (1) Remove all solutions in the reservoirs.
- (2) Power down the mixer controller.
- (3) Maintain a low gas pressure (so that one can still operate the mixer). Press the small red button on the No. 4 electromagnetic valve using a pointing object such as a hex wrench. The main solution passage valve will rotate to connect reservoirs to the syringes.
- (4) Shut off the gas bottle. Keep the small amount of the pressurized gas in the gas tubing.
- (5) Remove gas tubing from 1 through 4 in order. Observe that the solution passage valve does not rotate. The removal of a piece of tubing results in small amount of gas leakage that stops instantaneously as long as the main gas valve is shut. Refer to Chapter 5 for the instruction on how to remove gas tubing.
- (6) Loosen two set screws that hold the syringes.

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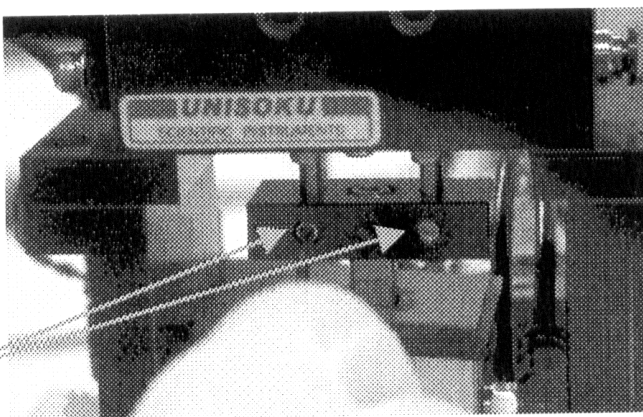


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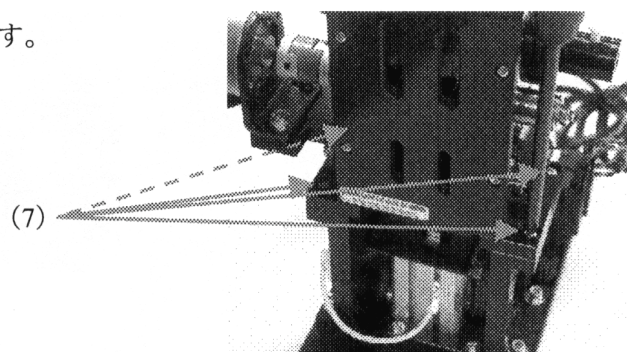
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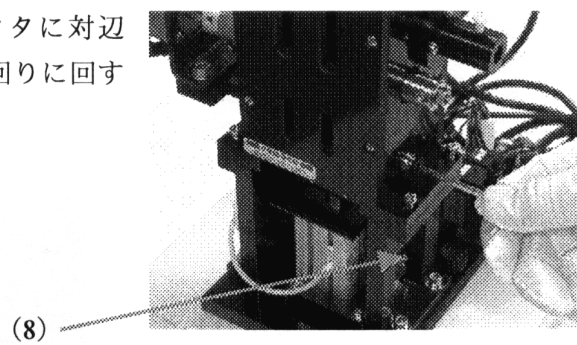
p21

- (7) Remove four screws with Phillips head.
- (8) Remove “flow joint”. Inset a 3mm hex wrench into the connector and rotate counter-clockwise to remove.
- (9) Hold the mixer base and lift the upper part to disengage the mixer body from the base.
- (10) Remove three screws with Phillips head from the underside of the mixer body, which you’ve just separated from the mixer base.

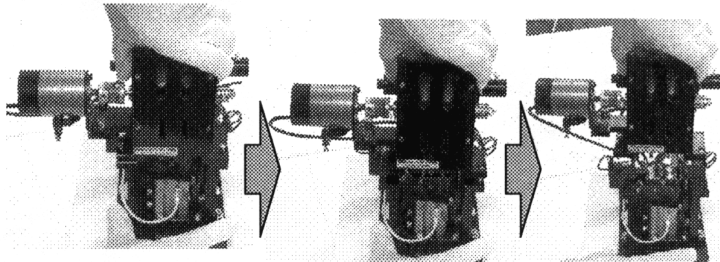
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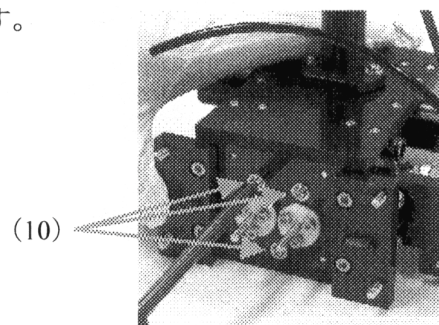
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- (11) Hold the plunger rods and remove syringes together with the place. Be sure to retain O-rings.
- (12) Mount the new O-ring(s) and syringe(s). Put an O-ring first and insert a syringe.
- (13) The plunger rods must be aligned properly to fit the hole on the Teflon piece.
Tighten three Phillips screws securely. Rotate the plunger rods so that the flat cut out faces front.
- (14) Press down the plunger holder at the top of the pneumatic cylinder.

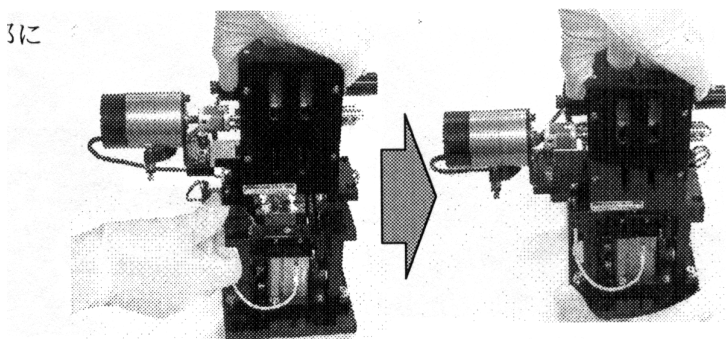


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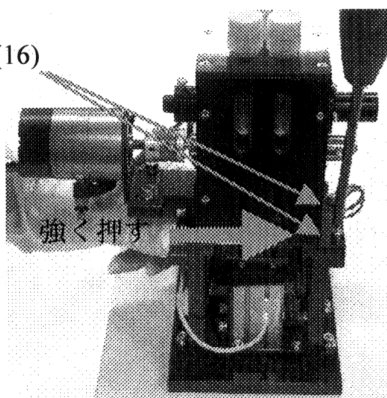
p23

- (15) Put back the mixer body onto the base.
- (16) Tighten two Phillips screws on the right side of the mixer while you press the mixer body to the right side (to the direction opposite from the rotory actuator.
- (17) Tighten other two Phillips screws. No longer need to keep pressure to the righ.
- (18) Put back “flow joint”.

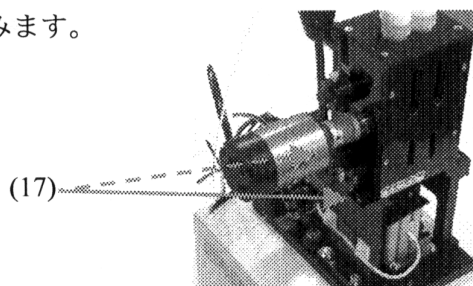
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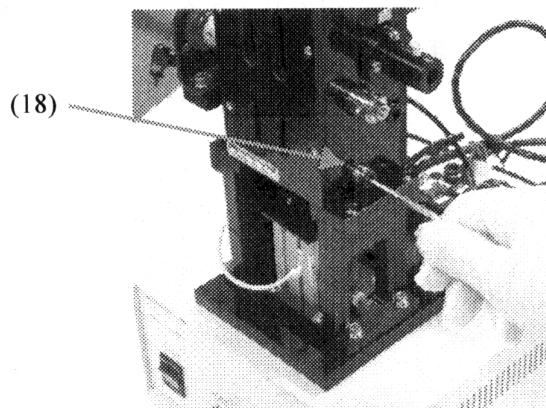
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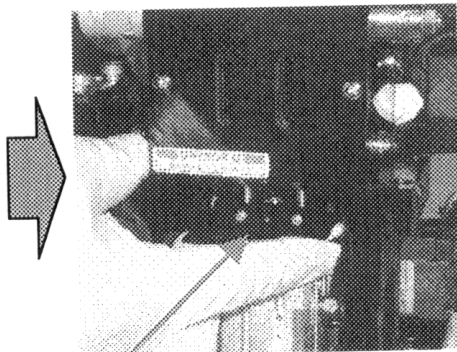
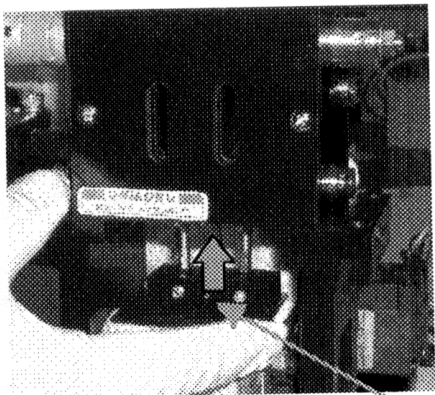


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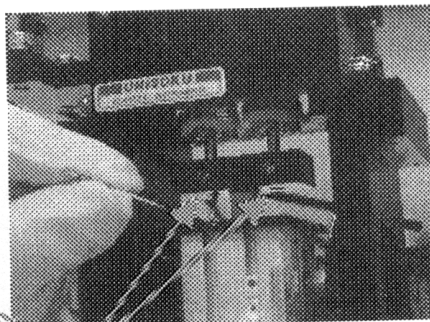
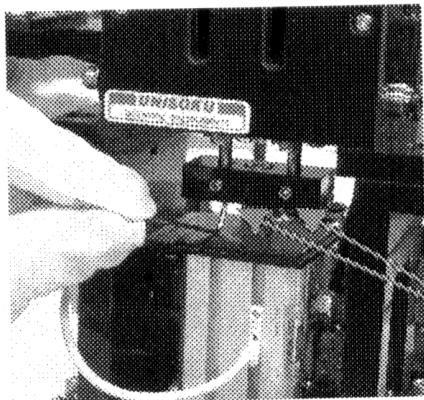
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- (19) Insert plunger rods into the holder by pushing up the plate above the pneumatic cylinder. Be sure to push it up all the way.
- (20) Push up both of two plunger rods using a hex wrench so that the plungers will make contact with the upper end of the syringes.
- (21) Be sure once again that the flat cut-out on the plunger faces front. Tighten two set screws to fix the rod position.



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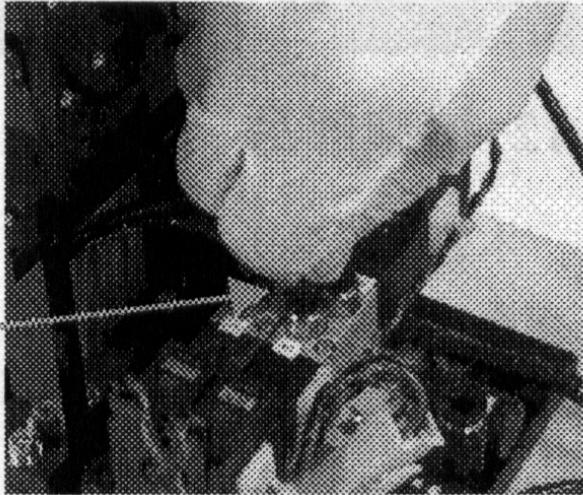
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- (22) Put back four pieces of gas tubing. Be sure to match indexes.
- (23) Open the main gas valve.
- (24) Power on the mixer controller.
- (25) Press "SET" button.
- (26) Press "MIX" button.

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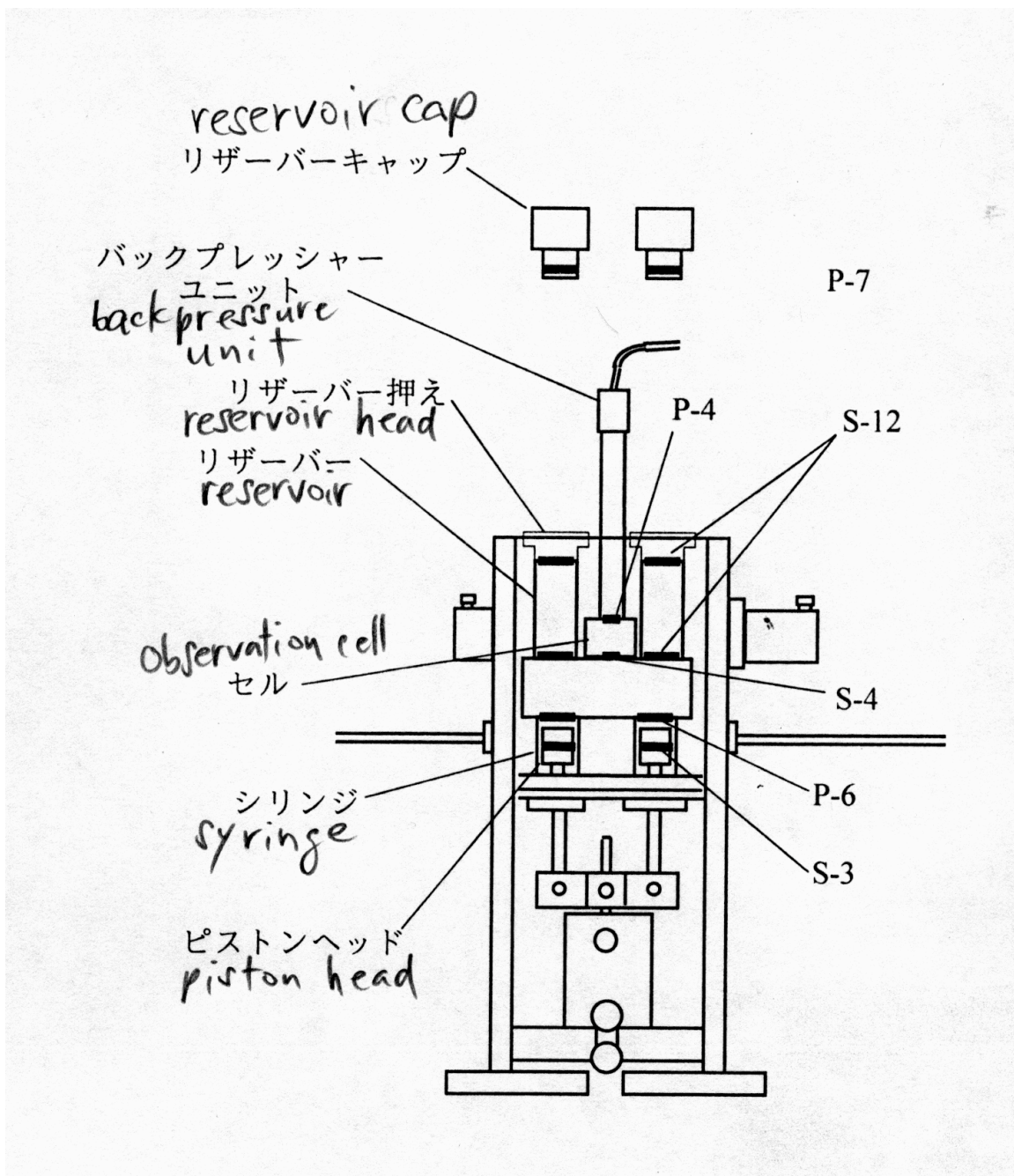
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12. Spare parts

One can replace O-rings by removing syringes, reservoirs, mixing chamber and observation cell. Refer to the diagram below for O-ring size.



Upper to lower:

Reservoir cap

Back pressure unit

Reservoir screw

Reservoir

Cell

Syringe

Piston head

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13. Chemicals compatibility.

1. Silicon O-rings

- acetaldehyde
- acetonitrile
- acetone
- ethanol
- ether
- dichloromethane
- xylene
- ?
- chloroform
- acetic acid
- ethylacetic acid
- diethylether
- tetrachlorocarbon
- cyclohexane
- tetrahydrofuran (?) (THF)
- tri-?
- toluene
- benzene
- formaldehyde
- methanol
- methylethylketone
- organic acid
- strong acid
- weak acid
- strong alkaline
- weak alkaline

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2. Teflon coated O-rings

acetaldehyde
acetonitrile
acetone (limited to Teflon head only)
ethanol
ether
dichloromethane
xylene
?
chloroform
tetrachlorocarbon
cyclohexane
acetic acid
ethylacetic acid
diethylether
tetrahydrofuran (?) (THF)
tri-?
toluene
benzene
formaldehyde
methanol
methyl ethyl ketone
hydrochloric acid
sulfuric acid
ammonia
calcium hydroxide
weak acid

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3. Viton O-rings

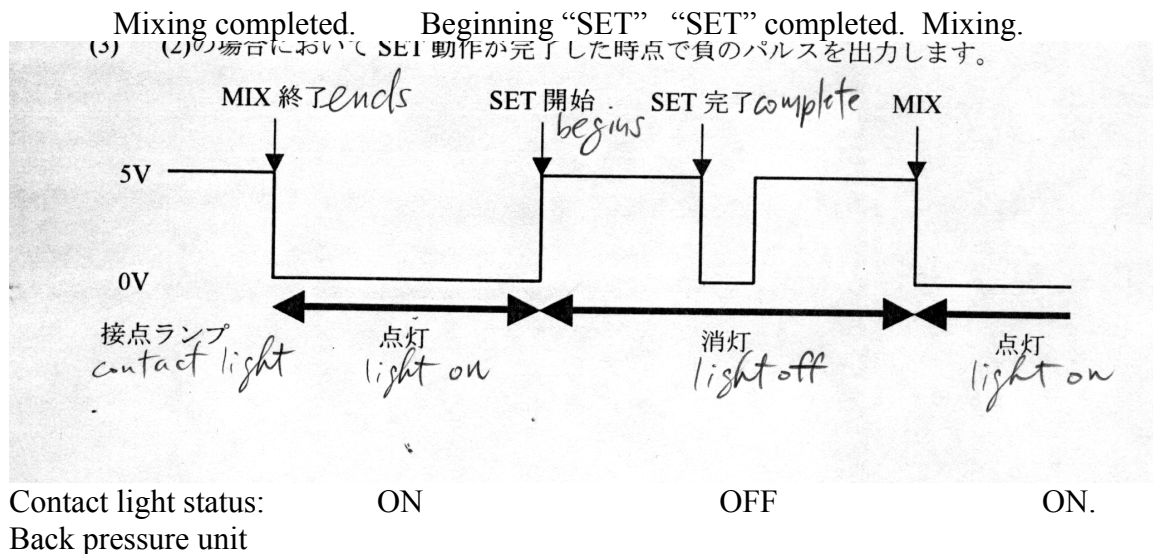
acetaldehyde
acetonitrile
acetone (limited to Teflon head only)
ethanol
ether
dichloromethane
xylene
?
chloroform
tetrachlorocarbon
cyclohexane
acetic acid
ethylacetic acid
diethylether
tetrahydrofuran (?) (THF)
tri-?
toluene
benzene
formaldehyde
methanol
methyl ethyl ketone
hydrochloric acid
sulfuric acid
ammonia
calcium hydroxide
weak acid

- Double circle symbol: No or very little damage
- Single circle symbol: Some damage, but sufficiently resistant, depending on actual condition.
- Triangle symbol: Avoid if possible.
- X symbol: Severe damage will result. Do not use.
- Minus symbol: No test result.

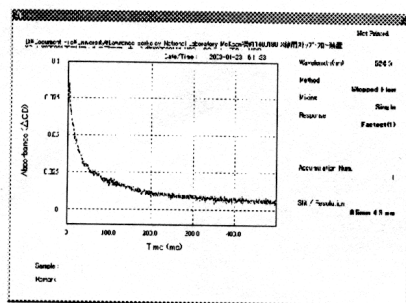
Supplement:

The three branching cables have following outputs:

- (1) When the Mix contact sensor light is on (duration since the syringe plunger has been pushed all the way till the plunger starts to be pulled), it gives 0V.
- (2) When the Mix contact sensor light is off (when the plunger has not been pushed) it gives 5V.
- (3) In the case of (2), it generates a negative pulse when the "SET" motion is completed.

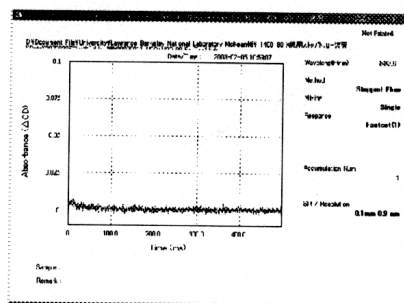


This instrument is equipped with a back-pressure unit to prevent "cavitation" effects. We observed a significantly large artificial (optical absorption) signal (OD ~0.1) due to the cavitation effects when we tested the instrument without the back-pressure unit. It effectively diminishes when the unit is equipped with the back pressure unit. We shipped the instrument with the back-pressure unit installed even though we just do not know whether a similar effect can be observed in x-ray signal. If the unit has undesirable effects in your measurements please remove.



バックプレッシャーユニット無し

Without back pressure unit.



バックプレッシャーユニット有り

With back pressure unit installed.